PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Case No. 02-134-D)

In application of	)	
A. Lavie, *et al.*	)	Examiner: Lei Yao
Serial No. 10/791,155	)	
Filed: March 1, 2004	)	Group Art Unit: 1642
For: Use of Specifically Engineered Enzymes to Enhance the Efficacy of Prodrugs	)	Confirmation No.: 3822

Mail Stop AF
Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-14501

Declaration Pursuant to 37 C.F.R. §1.312

I, Arnon Lavie, declare as follows:

1. I am one of the inventors of the above-mentioned application.
2. I am an associate professor at the Department of Biochemistry and Molecular Genetics, University of Illinois at Chicago. Attached as Exhibit A is my *curriculum vitae*.
3. I am knowledgeable in the field of molecular biology in general, and particularly in the art of human deoxycytidine kinase.
4. The physiological function of the salvage enzyme human deoxycytidine kinase (dCK) is to convert the nucleosides dC, dA, and dG into their corresponding monophosphates. Ultimately, the triphosphorylated forms of these nucleosides are substrates for DNA polymerases.
5. It has been shown that, under physiological conditions, native human dCK is mainly located in the cytoplasm and

phosphorylates deoxynucleosides in the cytoplasm. See Hatzis *et al*, 1998, JBC, Vol 273, pp 30239-30243. A copy of the reference is attached to the declaration as Exhibit B.

6. The result shown in Hatzis *et al*. is consistent with the current view that the nucleosides are phosphorylated in the cytoplasm by dCK and subsequently translocate to the nucleus to participate in DNA replication or repair. See Discussion on page 30241 of Exhibit B.

7. Hatzis *et al*. also showed that when the dCK protein is over-expressed in the cell by transfection, it is mainly located in the nucleus. (Compare Figure 2A and 2C of Exhibit B) Thus, any reported nuclear localization of transfected dCK is likely an artifact of over-expression.

8. Thus, I conclude that native dCK is predominantly located in the cytoplasm and performs its physiological function in the cytoplasm.

9. Further, I declare that the results depicted in Figures 6-9 of the specification demonstrate that the antibody-dCK conjugate enhances cell death in the presence of nucleoside analogs such as AraC.

10. Figure 6 of the specification shows that the combination of antibody HuM195-dCK conjugate and AraC increases the percentage of apoptotic cells compared to AraC alone. HuM195 binds specifically to CD33 antigen, which is expressed on the surface of HL60 and NB4 cells. About 50% of HL60 cells that were treated with AraC alone at a concentration of 1 μM for 96 hours underwent apoptosis (white bar in the "Cells + AraC (1μM)" group). Over 70% of the cells underwent apoptosis when the cells were treated with AraC and 10 μg of the antibody-dCK conjugate (white bar in the "Cells + AraC 1μM + Conj 10 μg" group). The increase in apoptosis (by

about 20%) is significant and is due to the presence of the conjugate.

11. Figure 7 of the specification shows that the combinatorial treatment of HL60 cells with the conjugate and AraC resulted in less cell survival than treatment of AraC alone as measured by trypan blue exclusion assay. Taken the 96 hr treatment for example, more than 80% of the HL60 cells survived after the AraC-alone treatment, whereas less than 10% of the cells survived after treatment with AraC and the HuM195-dCK conjugate.

12. Figure 8 of the specification shows that NB4 cells treated with HuM195-dCK conjugate and AraC exhibited increased apoptosis as compared to cells treated with AraC alone. About 40% of the NB4 cells that were treated with AraC alone for 96 hours underwent apoptosis (white bar in the "Cells + AraC (250nM)" group). The percentage of apoptotic cells increased to nearly 70% when cells were treated with a combination of HuM195-dCK conjugate and AraC (white bars in the "Cells + AraC + Con100 μg" group).

13. Finally, Figure 9 shows that the enhanced cell killing effect of the conjugate is specific in cells expressing the antigen recognized by the antibody within the conjugate. 293 cells do not express CD33 on the surface. See page 85 of the specification. Similar levels of cell death were observed, as expected, in cells treated with AraC alone or AraC and the conjugate, because the conjugate could not enter the cells and exert its effects.

14. In summary, the combinatorial treatment of AraC and the conjugate exerts an additional 20% (Figure 6), 30% (Figure 8), or 70% (Figure 7) cell death in CD33 positive cells compared to AraC

alone. Thus, I conclude that the enhanced effect on cell killing exerted by the antibody-dCK conjugate is significant.

15. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

Date: Sept 20, 2007

A. Lavie

Arnon Lavie, Ph.D.